entation of β-cell antigens to CD4+ and CD8+ T cells of non-obese diabetic mice. *J. Immunol.*, 151, 1723-1730.

Sprent, J, Lo, D., Gao, K. & Ren, Y.Y. (1988), T cell selection in the tymus. *Immunol. Rev.*, 101, 173-190.

Stern, L.J., Brown, J.H., Jardetzky, T.S., Gorga, J.C., Urban, R.G., Strominger, J.L. & Wiley, D.C. (1994), Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* (Lond.), 368, 215-221.

Todd, J.A., Bell, J.I. & McDevitt, H.O. (1987), HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* (Lond.), 329, 599-604.

Ziegler, H. K & Unanue, E.R. (1981), Identification of a macrophage antigen processing event required for I-region-restricted antigen presentation to T lymphocytes. *J. Immunol.*, 127, 1869-1875.

Ziegler, H. K & Unanue, E.R. (1982), Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. *Proc. Natl. Acad. Sci.* USA, 79, 175-178.

Mechanisms underlying the loss of self tolerance in NOD mice

M.A. Atkinson

Department of Pathology, The University of Florida, Gainesville FL 32610 (USA)

Insulin-dependent diabetes mellitus (IDDM) in both humans and NOD mice results from an autoimmune destruction of the insulin-producing pancreatic β cells (Atkinson and Maclaren, 1994). Characterizing the T cells that mediate this autoimmune destruction, identifying the β-cell autoantigens which serve as targets in this response, and uncovering the genes that underline the susceptibility to this disease all comprise matters of intense research investigation and controversy. This commentary, however, focuses on investigations in the NOD mouse aimed at revealing the immunogenetic mechanisms that underlie the loss of self tolerance: a factor at the genesis of this disease and autoimmunity in general.

Mechanisms for the generation of self tolerance

Neither major histocompatibility complex (MHC) class I nor class II molecules have an inherent capacity to discriminate between peptides derived from foreign pathogens or normal endogenous proteins (von Boehmer and Kisielow, 1990). Therefore, in order to prevent the development of deleterious autoimmune responses, it is necessary to destroy or inactivate any T cells that express a rearranged T-cell receptor (TCR) that recognizes endogenous peptides bound to self MHC molecules. This process occurs through several mechanisms which select cells from the theoretical total pool of (approximately) 109 TCR clonotypes; a subset of effectors that can respond to foreign, but not endogenous peptides bound to self MHC molecules.

One such tolerogenic mechanism occurs in the thymus (von Boehmer and Kisielow, 1990, Blackman *et al.*, 1990). The TCR molecules of T-cell precursors differentiating within the thymus interact with peptides presented by MHC gene products expressed on both thymic epithelial cells and haematopoietically-derived antigen-presenting cells (APCs). These interactions result in the positive selection of T cells capable of recognizing foreign antigens presented by self MHC gene products. Immature T cells whose TCR engages endogenous peptides bound to self MHC molecules are normally negatively selected in the thymus through an

Received June 16, 1997.

activation-driven cell death process termed apoptosis. Several recent studies have demonstrated that the threshold of T-cell activation required to induce negative selection in the thymus is quantitatively greater than that required for positive selection (Ashton-Rickhardt *et al.*, 1994, Sebzda *et al.*, 1994).

The activities of autoreactive T cells escaping intrathymic deletion are suppressed in the periphery by immunoregulatory T cells which also must be stimulated by a highly activated APC in order to become functional (Ishikura *et al.*, 1989). However, not all autoreactive T cells can undergo apoptotic cell death in the periphery when stimulated to a sufficiently high activation threshold by APC presenting large quantities of the appropriate antigen (Rocha and Von Boehmer, 1991; Zhang *et al.*, 1992, Ucker *et al.*, 1992; Critchfield *et al.*, 1994). Another mechanism by which APCs can downregulate autoimmune responses is by inducing a change in the cytokine profile produced by $CD4^+$ T cells reacting against self peptides (Rabinovitch 1994; Paul and Seder, 1994; Liblau *et al.*, 1995). Autoimmune tissue destruction appears to be promoted when self-peptide-reactive $CD4^+$ T cells produce a Th1 pattern of cytokines including interleukin-2 (IL2) and interferon-γ (IFNγ). These Th1 cytokines amplify cytotoxic $CD8^+$ T-cell functions, as well as supporting macrophage activation and delayed-type hypersensitivity (DTH) responses. In contrast, autoimmune tissue destruction appears to be blocked when self-peptide-reactive $CD4^+$ T cells produce a Th2 pattern of cytokines including IL4, IL5, IL6, IL10 and IL13 which provide help for the activation of B-lymphocyte-mediated humoral immunity. Of these cytokines, IL4 appears to be most important is switching T cells from a Th1 to Th2 response profile. Generally, $CD4^+$ T cells switch from a Th1 to a Th2 profile as a function of increasing antigen dose presented by APC. The cytokine profile produced by $CD4^+$ T cells can also vary depending upon the type of APC providing antigenic stimulation, with B lymphocytes tending to promote the activation of Th2 responses. The particular MHC class II gene product that an APC uses to present a given antigen can also determine whether a Th1 or Th2 $CD4^+$ T-cell response is elicited (Murray *et al.*, 1993). These T-cell response patterns can also be effected by APC-produced cytokines, with macrophage production of IL12 or IL1 promoting Th1 and Th2 activation, respectively (Greenbaum *et al.*, 1988; McAuthor and Raulet, 1993; Germann *et al.*, 1993; Taylor-Robinson and Phillips, 1994).

A constant for all of these immunoregulatory mechanisms is that high levels of T-cell stimulation tend to promote tolerance, while lower levels tend to promote immunological effector responses. Therefore, any genetic defects that compromise the stimulatory capacity of APC and/or impair T-cell responsiveness could preferentially diminish any or all of these tolerogenic mechanisms without fully abrogating immunological effector responses. As discussed for the remainder of this commentary, such defects are present in both APC and T cells from NOD mice and may be central to the development of autoimmune IDDM.

Immunoregulatory dysfunctions in NOD mice

As covered elsewhere in this Forum, the spectrum of T-cell clonotypes associated with autoimmune β-cell destruction in NOD mice is broad and includes the generation of cells capable of recognizing many self antigens (e.g., glutamic acid decarboxylase (GAD), insulin, heat shock protein, etc.). The presence of these autoreactive cells represents a failure of the aforementioned immunoregulatory mechanisms aimed at eliminating self-reactive T cells (i.e., physical deletion either in the thymus or periphery, functional suppression by immunoregulatory T cells, or a non-pathogenic rendering through a shift in their cytokine production from a Th1 to Th2 profile). For each of these immunoregulatory mechanisms, the threshold of T-cell activation required to induce tolerance is higher than that required to trigger an effector response. Hence, defects that compromise the stimulatory capacity of APC and/or impair T-cell responsiveness could preferentially diminish tolerogenic mechanisms without fully abrogating immunological effector functions. As these immunoregulatory dysfunctions are present in both APC and T cells from NOD mice, they may be major contributors to the development of autoimmune IDDM.

MHC-related dysfunctions

The unusual MHC haplotype of NOD mice (i.e., $H2^{g7}$) contributes to several APC dysfunctions that may lead to the development of β-cell autoreactive T cells. Diabetes rarely develops (i.e., < 3% incidence) in congenic stocks of NOD mice that heterozygously express MHC haplotypes from other strains (Prochazka *et al.*, 1989; Wicker *et al.*, 1989, 1992). Therefore, the immunotolerogenic defects that underlie the development of β-cell autoreactive T cells in NOD mice occur most readily when $H2^{g7}$ is homozygously expressed. One potential explanation for this observation is that $H2^{g7}$ MHC molecules are unable to present antigens in an efficient fashion to T-cell clonotypes

with potential β-cell autoreactivity. Such an activity could result in the preferential activation of effector rather than immunotolerogenic functions. Supporting this hypothesis is the recent demonstration that the αβ chain complexes which comprise I-Ag7 MHC class II molecules in NOD mice do not dimerize in a stable fashion (Carrasco-Marin *et al.*, 1996). This instability results in a marked decrease in the efficiency by which these MHC molecules bind and present antigen.

Another immunotolerogenic defect associated with homozygous expression of the $H2^{g7}$ MHC haplotype is the reduced ability of NOD APC to activate immunoregulatory T cells in a syngeneic mixed leukocyte reaction (SMLR) (Serreze and Leiter, 1988, 1991a). Multiple lines of evidence suggest that the failure to activate immunoregulatory T cells in an SMLR contributes to the pathogenesis of diabetes in NOD mice. First, the SMLR defect is overridden and IDDM inhibited in NOD mice treated with recombinant IL2 (Serreze *et al.*, 1990). Second, IDDM is diminished in NOD mice injected at a young age with cloned lines of immunoregulatory T cells propagated from a SMLR supplemented with IL2 *in vitro* (Reich *et al.*, 1989; Akhtar *et al.*, 1995; Choisch *et al.*, 1993).

The reduced ability of NOD mice to produce immunoregulatory T cells that block the function of diabetogenic effectors appears to result from homozygous expression of the unusual $I\text{-}A^{g7}$ MHC class II gene product. This concept is supported by the observation that diabetes is inhibited in NOD mice that transgenically express other I-A MHC class II molecules (Miyazaki *et al.*, 1990; Lund *et al.*, 1990; Slattery *et al.*, 1990), and that T cells from such animals can passively transfer disease resistance (Singer *et al.*, 1993). The development of IDDM is also blocked in transgenic NOD mice with restored I-E MHC class II expression on their APC (Lund *et al.*, 1990; Uno *et al.*, 1996; Hanson *et al.*, 1996). Therefore, in addition to those brought about by the presence of $I\text{-}A^{g7}$ molecules, some immunoregulatory dysfunctions which underlie the development of β-cell autoreactive T cells in NOD mice are most likely caused by their failure to express an I-E MHC class II gene product on APC. Indeed, the restoration of I-E expression on APC may block IDDM development in NOD mice by inducing a Th1 to Th2 cytokine shift by $CD4^+$ T cells responding to the β-cell autoantigens (e.g., GAD) (Hanson *et al.*, 1996).

While transgenic expression of an I-A or I-E class II gene product derived from a diabetes-resistant MHC haplotype restores the ability of NOD APC to activate many immunoregulatory mechanisms, they remain incapable of mediating clonal deletion of β-cell autoreactive T cells (Parish *et al.*, 1993; Slattery *et al.*, 1993). However, utilizing a competitive bone marrow reconstitution system, β-cell autoreactive T cells which normally develop from NOD bone marrow are deleted when forced to mature in the presence of APC from a stock of NOD mice that congenically express an MHC haplotype associated with IDDM resistance (Serreze and Leiter, 1991b). This may result from the protective APC population expressing class I as well as class II gene products from a diabetes-resistant MHC haplotype. Collectively, these results indicate that as APC express increasingly larger numbers of genes from a diabetes-resistant MHC haplotype, they acquire the ability to activate a wider array of immunotolerogenic mechanisms that limit both the generation and function of β-cell autoreactive T cells.

Non-MHC related dysfunctions

Non-MHC controlled defects also contribute to the reduced ability of NOD APC to activate immunoregulatory functions. These non-MHC-controlled defects appear to include a reduced ability of NOD APC to provide T-cell costimulatory signals at levels sufficient to induce tolerogenic rather than effector functions.

One factor contributing to the reduced T-cell costimulatory capacity of APC from NOD mice is that macrophages from these animals produce significantly less IL1 than those from IDDM-resistant strains (Serreze and Leiter, 1988; Jacob *et al.*, 1990). The reduced ability of NOD macrophages to produce IL1 results from their failure to fully differentiate from precursor cells in bone marrow (Serreze *et al.*, 1993a; Langmuir *et al.*, 1993; Serreze *et al.*, 1993b) This defect may be of pathogenic significance, since IDDM is blocked in NOD mice treated with IL1 *in vivo* (Jacob *et al.*, 1990). The mechanism behind this protection from IDDM may derive from studies indicating that IL1 supplementation *in vitro* restores the ability of NOD APC to activate regulatory T cells in an SMLR (Serreze and Leiter, 1988). Another, possibility is that this protection from IDDM results from the ability of IL1 to preferentially activate a Th2 rather than a Th1 response by $CD4^+$ T cells (McAuthor and Raulet, 1993; Taylor-Robinson and Phillips, 1994).

An additional factor contributing to the reduced T-cell costimulatory capacity of APC from NOD mice is that their macrophages produce unusually high levels of prostaglandin E-2 (PGE2) (Robinson *et al.*, 1993). This characteristic could contribute to the development of β-cell autoreactive T cells via several mechanisms. Purified dendritic cells from NOD mice represent a highly efficient APC popu-

lation for activating immunoregulatory T cells in an SMLR; however, this function is normally suppressed by the high levels of PGE2 produced by macrophages (Robinson *et al.*, 1993). PGE2 can also downregulate macrophage function in an autocrine fashion by elevating intracellular cAMP levels which in turn antagonize the protein kinase C (PKC)-mediated second messenger pathways required for IL1 secretion (Dinarello, 1988; Cheung and Hamilton, 1992; Bakouche *et al.*, 1992; Shapira *et al.*, 1994). As described above, the resulting decrease in IL1-mediated T-cell costimulatory activity could then block the induction of various immunoregulatory functions. In similar fashion, a PGE2-induced elevation of intracellular cAMP can block the induction of immunoregulatory mechanisms directly at the T-cell level by antagonizing TCR-coupled PKC second messenger activities. Indeed, the presence of PGE2 has been shown to inhibit the ability of T cells to be driven to an activation state sufficient to induce apoptotic cell death following TCR cross-linking (Goetzl *et al.*, 1995).

Correcting MHC/non-MHC immunodysfunctions

If diminution of APC functions brought about by synergistic interactions between MHC and non-MHC genes is truly central to the pathogenesis of autoimmune IDDM, this may provide an explanation for the seemingly paradoxical finding that IDDM is inhibited in NOD mice treated with a wide range of non-specific immunostimulatory agents (Rabinovitch *et al.*, 1994; Singh and Rabinovitch, 1993; Bowman *et al.*, 1994). Indeed, we have previously suggested that one possible approach for IDDM prevention in humans could be the use of non-specific immunostimulatory agents to upregulate production of T-cell costimulatory factors by APC (Bowman *et al.*, 1994). In addition, the reduced ability of $H2^{g7}$ MHC molecules to present β-cell autoantigens in a manner efficient enough to induce tolerogenic rather than effector T-cell response can be overridden by exposing NOD APC to large quantities of these antigens. This contention was demonstrated by the studies indicating that the development of β-cell autoreactive T cells and IDDM are both inhibited in NOD mice intrathymically injected at a young age with whole syngeneic islet cells as well as the β-cell autoantigen GAD (Tisch *et al.*, 1993; Gerling *et al.*, 1992) Overriding inefficient presentation of β-cell autoantigen by $H2^{g7}$ MHC molecules could also account for the finding that IDDM development is inhibited in NOD mice injected with dendritic cells purified from pancreatic lymph nodes (presumably through presentation of high-levels of β-cell antigens), but not from cervical or axilliary nodes (Clare-Salzler *et al.*, 1992). Also supporting the concept that $H2^{g7}$-positive APC can be made tolerogenic when pulsed with high-doses of β-cell autoantigens are studies indicating that NOD T cells isolated from pancreatic lymph nodes are less efficient than those obtained from other anatomical sites in passively transferring IDDM (Lepault *et al.*, 1993).

Evidence does exist to suggest that some immunotolerogenic defects in NOD mice are intrinsic to T cells. Specifically, NOD thymocytes proliferate poorly in response to TCR cross-linking agents (Zipris *et al.*, 1991). Subsequent studies demonstrated that this defect results from a reduced ability of NOD thymocytes to activate TCR-coupled PKC second messenger pathways (Rapoport *et al.*, 1993). This could contribute to the development of IDDM by inhibiting the ability of immature T cells with potential β-cell autoreactivity to be driven to an activation state sufficient to induce their deletion via apoptosis. Support for this possibility is provided by the finding that the NOD thymoctye response defect is at least partially controlled by a gene mapping to the region of chromosome 11 previously shown to contain the diabetes susceptibility locus *Idd4* (Gill *et al.*, 1995).

Conclusions

NOD mice generate a diverse array of both MHC class I- and class II-restricted T-cell clonotypes capable of initiating and then amplifying autoimmune destruction of pancreatic β cells. While apparently more limited in scope than the T-cell clonotypes that respond to them, a relatively broad spectrum of β-cell antigens are targets of the autoimmune response in NOD mice. Therefore, were a similar situation to exist in humans, it may prove difficult to develop IDDM prophylactic therapies targeted to "the diabetogenic T-cell clonotype" or "the β-cell autoantigen". Studies in NOD mice do, however, indicate that it may be possible to prevent IDDM with therapies that correct defects which underlie the genesis of β-cell autoreactive T cells. Paradoxically, it appears that these therapies would have to be immunostimulatory in nature; a treatment which enables T cell/APC interactions to occur at levels vigorous enough to preferentially activate tolerogenic rather than effector functions. If investigations of humans continue to reveal similar mechanisms of immune dysfunctions in persons at increased risk for IDDM (e.g., enhanced PGE2, reduced IL4, abnormal CTLA-4/CD40, etc.), the initiation of clinical trials aimed at their correction may be warranted in the hope of disease prevention.

References

Akhtar, I., Gold, JP., Pan, L.-Y. *et al.* (1995), CD4+ β islet cell-reactive T cell clones that suppress autoimmune diabetes in nonobese diabetic mice. *J. Exp. Med.*, 182, 87-97.

Ashton-Rickardt, P.G., Bandeira, A., Delany, J.R. *et al.* (1994), Evidence for a differential avidity model of T cell selection in the thymus. *Cell.*, 76, 651-663.

Atkinson, M., Maclaren, N. (1994), The pathogenesis of insulin dependent diabetes mellitus. *N. Engl. J. Med.*, 331, 1428-1436.

Bakouche, O., Moreau, J.L., Lachman L.B. (1992), Secretion of IL-1: role of protein kinase C. *J. Immunol.*, 148, 84-91.

Blackman, M., Kappler, J., Marrack, P. (1990), The role of the T cell receptor in positive and negative selection of developing T cells. *Science*, 248, 1335-1341.

Bowman, M.A., Leiter, E.H., Atkinson, M.A. (1994), Prevention of diabetes in the NOD mouse: implications for therapeutic interventions in humans. *Immunol. Today*, 15, 115-120.

Carrasco-Marin, E., Shimizu, J., Kanagawa, O. &Unanue, ER. (1996), The class II MHC class I-A^{g7} molecules from non-obese diabetic mice are poor peptide binders. *J. Immunol.*, 156, 450-458.

Cheung, D.L., Hamilton, J.A. (1992), Regulation of human monocyte DNA synthesis by colony stimulating factors, cytokines, and cyclic adenosine monophosphate. *Blood*, 79, 1972-1981.

Choisch, N. & Harrison, L.C. (1993), Suppression of diabetes mellitus in the non-obese diabetic (NOD) mouse by an autoreactive (anti-I-A^{g7}) islet-derived CD-4+ T-cell line. *Diabetologia*, 36, 716-721.

Clare-Salzler, M.J., Brooks, J., Chai, A., Herle, K.V. & Anderson, C. (1992), Prevention of diabetes in non-obese diabetic mice by dendritic cell transfer. *J. Clin. Invest.*, 90, 741-748.

Critchfield, J.M., Racke, M.K., Zuniga-Pflucker, J.C. , *et al.* (1994), T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science*, 263, 1139-1143.

Dinarello, C.A. (1988), Biology of interleukin 1. *FASEB J.*, 2, 108-115.

Gerling, I.C., Serreze, D.V., Christianson, S.W. & Leiter, E.H. (1992), Intrathymic islet cell transplantation reduces beta-cell autoimmunity and prevents diabetes in NOD/Lt mice. *Diabetes*, 41, 1672-1676.

Germann, T., Gately, M.K., Schoehaut, D.S., *et al.* (1993), Interleukin-12/T cell stimulating factor, a cytokine with multiple effects on T helper 1 (Th1) but not on Th2 cells. *Eur. J. Immunol.*, 23, 1762-1770.

Gill, B.M., Jaramillo, A., Ma, L., Laupland, K.B. & Delovitch, T.L. (1995), Genetic linkage of thymic T-cell proliferative unresponsiveness to mouse chromosome 11 in NOD mice. *Diabetes*, 44, 614-619.

Goetzl, E.J., An, S. & Zeng, L. (1995), Specific suppression by Prostaglandin E2 of activation induced apoptosis of human CD4+8+ T lymphoblasts. *J. Immunol.*, 154, 1041-1047.

Greenbaum, L.A., Horowitz, J.B., Woods, A., Pasqualini, T., Reich, E.P. & Bottomly, K. (1988), Autocrine growth of CD4+ T cells: differential effects of IL-1 on helper and inflammatory T cells. *J. Immunol.*, 140, 1555-1560.

Hanson, M.S.,Cetkovic-Cvrlje, M., Ramiya, V.K., Atkinson, M.A. *et al.* (1996), Quantitative thresholds of MHC class II I-E expressed on hematopoietically derived APC in transgenic NOD/Lt mice determine level of diabetes resistance and indicate mechanism of protection. *J. Immunol.*, 157, 1279-1287.

Ishikura, H., Jayaraman, S., Kuchroo, V., Diamond, B., Saito, S. & Dorf, M.E. (1989), Functional analysis of cloned macrophage hybridomas. VII. Modulation of suppressor T cell-inducing activity. *J. Immunol.*, 143, 414-419.

Jacob, C.O., Aiso, S., Michie, S.A., McDevitt, H.O. & Acha-Orbea H.(1990), Prevention of diabetes in non-obese diabetic mice by tumor necrosis factor (TNF); similarities between TNF-a and interleukin 1. *Proc. Natl. Acad. Sci.* USA, 87, 968-972.

Langmuir, P., Bridgett, M., Bothwell, A. & Crispe I. (1993), Bone marrow abnormalities in the non-obese diabetic mouse. *Int. Immunol.*, 5, 165-177.

Lepault, F., Faveeuw, C., Luan, J.J. & Gagnerault, M.C. (1993), Lymph node T-cells do not optimally transfer diabetes in NOD mice. *Diabetes*, 42, 1823-1828.

Liblau, R.S., Singer, S.M. & McDevitt, H.O. (1995), Th1 and Th2 CD4+ T cells in the pathogenesis of organ specific autoimmune diseases. Immunol. *Today*, 16, 34-38.

Lund, T., O'Reilly, L., Hutchings, P. *et al.* (1990), Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A fl-chain or normal I-E a-chain. *Nature*, 345, 727-729.

McAuthor, J.G. & Raulet D.H. (1993), CD28-induced costimulation of T helper type 2 cells mediated by induction of responsiveness to interleukin 4. *J. Exp. Med.*, 178, 1645-1653.

Miyazaki, T., Uno, M., Uehira, M. *et al.* (1990), Direct evidence for the contribution of the unique I-Anod to the development of insulitis in non-obese diabetic mice. *Nature* (Lond.), 345, 722-724.

Murray, J.S., Madri, J., Pasqualini, T. & Bottomly, K. (1993), Functional CD4 T cell subset interplay in an intact immune system. *J. Immunol.*, 150, 4270-4276.

Parish, N.M., Chandler, P., Quartey-Papafio, R., Simpson, E. & Cooke, A. (1993), The effect of bone marrow and thymus chimerism between non-obese diabetic (NOD) and NOD-E transgenic mice, on the expression and prevention of diabetes. *Eur. J. Immunol.*, 23, 2667-2675.

Paul, W.E. & Seder, R.S. (1994), Lymphocyte responses and cytokines. *Cell*, 76, 241-251.

Prochazka, M., Serreze, D.V., Worthen, S.M. & Leiter, E.H. (1989), Genetic control of diabetogenesis in NOD/Lt mice: development and analysis of congenic stocks. *Diabetes*, 38, 1446-1455.

Rabinovitch, A. (1994), Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM: therapeutic intervention by immunostimulation? *Diabetes*, 43, 613-621.

Rapoport, M.J., Lazurus, A.H., Jaramillo, A., Speck, E. & Delovitch, T.L. (1993), Thymic T cell anergy in autoimmune nonobese diabetic mice is mediated by deficient T cell receptor regulation in the pathway of p21ras activation. *J. Exp. Med.*, 177, 1221-1226.

Reich, E.P., Scaringe, D., Yagi, J., Sherwin, R.S. & Janeway, C.A. (1989), Prevention of diabetes in NOD mice by injection of autoreactive T lymphocytes. *Diabetes*, 38, 1647-1651.

Robinson, P., Cai, B., Keenan, C. & Clare-Salzler, M. (1993), Macrophage products mediate suppression of the syngeneic mixed lymphocyte response in NOD mice. *Autoimmunity*, 15 (suppl.), 49.

Rocha, B. & Von Boehmer, H. (1991), Peripheral selection of the T cell repertoire. *Science*, 251, 1225-1231.

Sebzda, E., Wallace, V.A., Mayer, J., Yeung, R.S.M., Mak, T. & Ohashi, P.S. (1994), Positive and negative thymocyte selection induced by different concentrations of a single peptide. *Science*, 263, 1615-1618.

Serreze, D.V. & Leiter, E.H. (1988), Defective activation of T suppressor cell function in Nonobese Diabetic mice. Potential relation to cytokine deficiencies. *J. Immunol.*, 140, 3801-3807.

Serreze, D.V., Hamaguchi, K. & Leiter, E.H. (1990), Immunostimulation circumvents diabetes in NOD/Lt mice. *J. Autoimmun.*, 2, 759-776.

Serreze, D.V. & Leiter, E.H. (1991a), Defective suppressor T cell activation in NOD/Lt mice is associated with pathogenesis and homozygous expression of H-2g7. *Diabetes*, 40 (suppl. 1), 49A.

Serreze, D.V. & Leiter, EH. (1991b), Development of diabetogenic T cells from NOD/Lt marrow is blocked when an allo-H-2 haplotype is expressed on cells of hematopoietic origin, but not on thymic epithelium. *J. Immunol.*, 147, 1222-1229.

Serreze, D.V., Gaskins, H.R. & Leiter, E.H. (1993a), Defects in the differentiation and function of antigen presenting cells in NOD/Lt mice. *J. Immunol.*, 150, 2534-2543.

Serreze, D.V., Gaedeke, J.W. & Leiter, E.H. (1993b), Hematopoietic stem cell defects underlying abnormal macrophage development and maturation in NOD/Lt mice: defective regulation of cytokine receptors and protein kinase C. *Proc. Natl. Acad. Sci.* USA, 90, 9625-9629.

Shapira, L., Takashiba, S., Champagne, C., Amar, S. & Van Dyke, T.E. (1994), Involvement of protein kinase C and protein tyrosine kinase in lipopolysaccharide-induced TNF-a and IL-1fl production by human monocytes. *J. Immunol.*, 153, 1818-1823.

Singer, S.M., Tisch, R., Yang, X.D. & McDevitt, H.O. (1993), An Abd transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. *Proc. Natl. Acad. Sci.* USA, 90, 9566-9570.

Singh, B. & Rabinovitch, A. (1993), Influence of microbial agents on the development and prevention of autoimmune diabetes. *Autoimmunity*, 15, 209-213.

Slattery, R.M., Kjer-Nielsen, L., Allison, J., Charlton, B., Mandel, T. & Miller, J.F.A.P. (1990), Prevention of diabetes in non-obese diabetic I-Ak transgenic mice. *Nature* (Lond.), 345, 724-726.

Slattery, R.M., Miller, J.F.A.P., Heath, W.R & Charlton, B. (1993), Failure of a protective major histocompatability complex class II molecule to delete autoreactive T cells in autoimmune diabetes. *Proc. Natl. Acad. Sci.* USA, 90, 10808-10810.

Taylor-Robinson, A.W. & Phillips, R.S. (1994), Expression of IL-1 receptor discriminates Th2 from Th1 cloned CD4+ T cells specific for Plasmodium chabaudi. *Immunology*, 81, 216-221.

Tisch, R., Yang, X.D., Singer, S.M., Liblau, R.S., Fugger, L. & McDevitt, H.O. (1993), Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature* (Lond.), 366, 72-75.

Ucker, D.S., Meyers, J. & Obermiller, P.S. (1992), Activation-driven T cell death. II. Quantitative differences alone distinguish stimuli triggering nontransformed T cell proliferation or death. *J. Immunol.*, 149, 1583-1592.

Uno, M., Miyazaki, T., Uehira, M., *et al.* (1991), Complete prevention of diabetes in transgenic NOD mice expressing I-E molecules. *Immunol. Lett.*, 31, 47-52.

von Boehmer, H. & Kisielow, P. (1990), Self-nonself discrimination by T cells. *Science*, 248, 1369-1373.

Wicker, L.S., Miller, B.J., Fischer, P.A., Pressey, A. & Peterson, L.B. (1989), Genetic control of diabetes and insulitis in the nonobese diabetic mouse. Pedigree analysis of a diabetic H-$2^{nod/b}$ heterozygote. *J. Immunol.*, 142, 781-784.

Wicker, L.S., Appel, M.C., Dotta, F. *et al.* (1992), Autoimmune syndromes in major histocompatability complex (MHC) congenic strains of nonobese diabetic (NOD) mice. The NOD MHC is dominant for insulitis and cyclophosphamide-induced diabetes. *J. Exp. Med.*, 176, 67-77.

Zhang, L., Martin, D.R., Fung-Leung, W.P., Teh, H.S. & Miller, R.G. (1992), Peripheral deletion of mature $CD8^+$ antigen specific T cells after in vivo exposure to male antigen. *J. Immunol.*, 148, 3740-3745.

Zipris, D., Lazarus, A.H., Crow, A.R., Hadazija, M. & Delovitch, T.L. (1991), Defective thymic T cell activation by concanavalin A and anti-CD3 in autoimmune nonobese diabetic mice: Evidence of thymic T cell anergy that correlates with the onset of insulitis. *J. Immunol.*, 146, 3763-3771.